

Partial Characterisation of *Salmonella gallinarum* Clinical Isolate and Expression of Its Antigenic Outer Membrane Protein C (OmpC) Gene In Planta

Ee Leen Pang¹, Hwei-San Loh^{1,2*}

¹School of Biosciences, Faculty of Science, The University of Nottingham Malaysia Campus, Jalan Broga, 43500 Semenyih, Selangor, Malaysia.

²Biotechnology Research Centre, The University of Nottingham Malaysia Campus, Jalan Broga, 43500 Semenyih, Selangor, Malaysia.

ABSTRACT

Fowl typhoid's epidemiology and disease intervention have been extensively studied since 1950's owing to its high mortality and morbidity rates. Even up-to-date, outbreaks are incessantly haunting poultry industries of major continents. *Salmonella gallinarum*, the etiologic agent of fowl typhoid, was used to develop a series of vaccination regime. However, treatments are gradually losing effectiveness due to residual virulence from mutated strains and rapid evolution of multi-drug resistance isolates. Hence, in planta subunit vaccine production is proposed to surpass current limitations. The homotrimeric osmoporin (outer membrane protein C) is a potent candidate antigen that confers momentous stimulation of humoral and cell-mediated immune responses in broilers. This research signified the potential development of a plant-expressed OmpC immunogen. The project scope embarked on the identification of *S. gallinarum* clinical isolate, construction of expression cassette and delivery of constructs into *Nicotiana benthamiana* via agroinfiltration. The OmpC transcripts and proteins were detected successfully at the molecular weights of ~1.002 kbp and ~35 kDa, respectively. These preliminary findings pave the feasibility of biomanufacturing a safe and cost-effective fowl typhoid vaccine that would confer multi-protection against other significant *Salmonella* infections attributed to the high sequence homology of the OmpC gene. Speed improvement is demonstrated and transient expression appears to outperform conventional platforms in expediting vaccine production for an emerging pandemic strain.

Keywords: *OmpC* gene, *KDEL* signal, *Salmonella gallinarum*, subunit vaccine

INTRODUCTION

Fuelled by the trend of increasing urbanisation, meat demands are progressively elevating where 29% increment is specifically pressured upon poultry meat production [1]. Accounting for almost half of world meat trading, figures of 9,700,000 and 10,500,000 tonnes were recorded in years 2004 and 2008, respectively [2,3]. Poultry products which serve as cheap source of protein are now widely known to be threatened by *Salmonella* diseases [4]. Therefore, in the course of demanding increment for international trading, the risk of global dissemination of poultry infections is concurrently exacerbated. In fact, huge invest-

ments have already been laid down by European countries in salmonellosis disease control. Hitherto, series of molecular diagnostics tools have been deployed for rapid serotyping of clinical isolates such as real-time multiplex polymerase chain reaction (qPCR) assay [5]. Nevertheless, more constructive approaches are anticipated; of which extensive researches are currently on-going to pave a way for efficient subunit vaccines production that takes consumer safety concerns into account. It is because the issue is previously neglected in terms of antibiotics and live-attenuated strain vaccines application [6]. Pathogenicity of host-adapted *Salmonella gallinarum* is primarily noted through the epidemic spread of fowl typhoid (FT) in chickens and turkeys, often instigating high morbidity and mortality rates. The first outbreak of the infectious septicaemia disease was reported back in England during 1888. Blood poisoning usually leads to enlargement of liver and spleen, sometimes with signs of white necrotic foci

*Corresponding author:

Hwei-San Loh

School of Biosciences, Faculty of Science, The University of Nottingham Malaysia Campus, Jalan Broga, 43500 Semenyih, Selangor, Malaysia

E-mail: Sandy.Loh@nottingham.edu.my

in other organs of the infected poultry [7,8]. Albeit eradication programme had been successful in Europe and other Western countries via vaccination and surveillance programmes, but severe losses were suffered by pandemic outbreaks in Asia, Latin America and Africa [9,10]. The severity of FT was highlighted when a total of 227 disease outbreaks were detected in Haryana alone simply within a 4-year period, affecting 9 out of 20 districts [11]. Death rate of acute FT can range from 10-93% [12]. Without underestimating the risk, 100% mortality rate had been reported in one-day-old broiler chicks after infected by 10^8 colony-forming unit/ml of *S. gallinarum* [13].

The upsurge of FT prevalence had pioneered the development of 9R live-attenuated vaccine with semi-rough lipopolysaccharide [14] that would not interfere with egg production and blood testing as compared to dead vaccines of no value. Dejectedly, minimal protection against highly virulent field strains and egg transmission of 9R strain were reported [15]. Because of natural variation, this is further complicated with the emergence of new strains that could hijack existing immune responses due to poor cross protection from the conventional vaccine. Albeit drugs application (i.e. chloramphenicol, tetracyclines and sulphonamides) are employed to control disease outspread, constant evolution of multi-drug resistant field strains has gradually hampered the effectiveness of eradication programmes. Dealing with residual virulence induced by whole cell bacteria and live strain vaccines, subunit vaccines development from immunodominant protein components were extensively researched for new vaccine formulation. Bacterial outer membrane protein is a potent inducer of both humoral and cell-mediated immune responses [16]. A remarkable breakthrough was achieved when administration of partially purified OmpC from *S. gallinarum* conferred superior protection as compared to whole cell-based and 9R strain live vaccines [17]. Selection of immunogen on the basis of long-lasting protection is vital. The trimeric β -barrel protein had satisfactorily fulfilled the criteria as 53-70% protection was passed on by immunised breeders to newly-hatched chicks after challenged with 20-500 half-maximum lethal dose of the pathogen [18].

With the popularised trend of exploiting plant systems as biofactories for the production of pharmaceutical products, this context aims to present a framework for rapid development of in planta vaccine candidate based on agroinfiltration strategy [19]. Hitherto, the transient expression platform has gained increasing adoption as a robust and efficient gene delivery

methodology for numerous biopharmaceutical proteins production. It is to serve as a prompt remedy to tackle conventional regime, which commonly entangles with more tedious works in the course of harvesting a safe and efficient vaccine for poultry at infested fields. Practically, plants stand out as a better alternative to displace prokaryotic and eukaryotic expression systems with improved efficiency, biocontainment warranty, higher safety and convenient storage. As plants are implicated not to harbour mammalian pathogens, this would then eliminate concerns of protein contamination with viruses, endotoxins or oncogenic sequences; correspondingly reduce the cost associated with screening and purification [20].

It is noteworthy to mention, the OmpC candidate antigen has never been isolated for heterologous expression in tobacco as described in this novel study. As the "old" vaccine is inadequate in combating new variants, continuous vaccine development is ultimately essential without being jeopardised by expensive and long lead time approach. Thereby, this paper reports the feasibility of a rapid vaccine development upon detection of an emerging outbreak. A clinical isolate of *Salmonella* disease was used here to demonstrate an industrial-like application in a safer and speedy production platform.

MATERIALS AND METHODS

Identification of Etiologic Agent

A clinical isolate of *S. gallinarum* obtained from an undisclosed local farm (Peninsular of Malaysia) was firstly subjected to morphological and biochemical identification. The colonial growth response was tested on several media such as Nutrient agar, MacConkey agar and Xylose-Lysine-Deoxycholate agar, using closely-related enterobacteria (*Escherichia coli*) for comparison. Differential staining methodology was used to confirm the presence of gram-negative rods under microscopic examination. The identity of the FT etiologic agent was reinforced through biochemical analyses on the utilisation of sugars and amino acids, motility observation and Analytical Profile Index (API) test.

Bacterial Genomic DNA Extraction and OmpC Gene Isolation

Following bacterial characterisation, intact genomic DNA (gDNA) of *S. gallinarum* was prepared via inuPREP Bacterial DNA Kit (Analytik Jena, Germany) as well as conventional means. For the latter, the adopted protocol [21] was modified correspondingly to

maximise gDNA template harvest. The extraction was conducted as follow: (i) lysis of 10 ml bacterial culture in buffer [40 mM Tris acetate (pH 7.8), 20 mM sodium acetate, 1 mM ethylenediaminetetraacetic acid (EDTA) and 1% sodium dodecyl sulphate (SDS)]; (ii) removal of cellular debris by adding 5M sodium chloride (NaCl); (iii) separate protein precipitates via chloroform treatment; (iv) ethanol precipitation and RNase A incubation; and (v) purification prior re-suspension in 1X TE buffer. Alongside, the primers targeted for amplification of full-length OmpC gene [0.969 kilobase pair (kbp)] were designed according to published sequence (Accession Number: NC_011274). For cloning purpose, NruI and XmaI restriction sites were introduced into 5' and 3' ends of the construct. Eukaryotic KDEL consensus sequence was also incorporated to the C-terminus of OmpC as product localisation signal. Detailed design of forward (PF1ompC) and reverse (PR1ompC) primers was specified in Figure 1a. A PCR cocktail was prepared by mixing reagents (1X PCR buffer, 1.5 mM MgCl₂, 0.3 mM dNTPs, 0.5 µM of each primer and 1.5 units/µl of Taq polymerase) with 100.0 ng/µl of extracted DNA template to isolate the antigenic gene. Optimised OmpC gene amplification was programmed as follow: (i) pre-denaturation step at 94°C; (ii) 30 amplification cycles with 94°C for denaturation, 67°C for annealing and 72°C for elongation; and lastly (iii) the final extension of amplicons at 72°C.

Recombinant Vector Construction

Amplicons collected from PCR were purified using Expin™ Combo GP (GeneAll®, Korea). As both pEAQ-HT vector (Accession Number: GQ497234) and OmpC insert had similar cutting site for NruI and XmaI, a two-step restriction endonuclease (RE) digestion was performed (Figure 1b). Subsequent ligation of digested fragments was performed via T4 DNA Ligase (New England Biolabs, UK) according to manufacturer's instructions. Heat-shock approach was then employed to introduce the recombinant vector, pEAQ::OmpC-KDEL into competent XL-1 *E. coli* cells. Putative recombinants were subjected to PCR-based screening to confirm the successful incorporation of the OmpC insert. Verification of positive clone was reinforced by extracting plasmids of transformed bacteria via Expres™ Plasmid Quick (GeneAll®, Korea) for RE and PCR analyses. To facilitate rapid screening, XhoI was used instead of XmaI since it shared common Buffer R as NruI. Upon confirmation, the extracted pEAQ::OmpC-KDEL was sent for nucleotide sequencing (Bioneer, Korea).

Plant Transformation via Agroinfiltration

Nicotiana benthamiana seeds were planted and grown up to 10-week old under controlled environment of 24°C. The recombinant vector, pEAQ::OmpC-KDEL was incorporated into competent *Agrobacterium tumefaciens* strain LBA4404 to establish stable transformant for downstream agroinfiltration. Broth cultures harbouring constructs of pEAQ::OmpC-KDEL, pEAQ::GFP and pEAQ-HT were grown for 48 hours at 28°C and 200 rpm continuous shaking condition. These agrobacterial cultures were then harvested at OD₆₀₀ = 0.4 by 4°C centrifugation (6,000 rpm for 10 minutes) and re-suspended in MMA solution [10 mM MES (2-[N-morpholino]ethanesulfonic acid) at pH 5.6, 10 mM magnesium chloride and 100 µM acetosyringone] [22]. Abaxial sides of tobacco expanded leaves were pricked before infiltrated with above mentioned suspensions using a needleless syringe. Three plant replicates were prepared for each construct along with a healthy tobacco as mock-infiltration control. Transformed plants were then allowed to resume growth and harvested at 3, 6 and 9 days post inoculation (dpi). Apparent morphological distortion of infected leaves was also recorded.

RNA Assessment of Infiltrated Leaves

To monitor expression and stability of foreign OmpC gene post-infiltration, TRIZOL® reagent (Invitrogen, USA) was used to extract the total RNA from 100 mg of grounded tobacco leaves [23] with liquid nitrogen. Phase separation was then achieved by 20% (v/v) chloroform treatment. Next, the extracted aqueous layer was subjected to precipitation by isopropanol and ethanol followed by elution of RNA in 100 mM Tris-HCl (pH 8.5). Two-step reverse-transcription PCR (RT-PCR) was performed to generate cDNA samples using Omniscript RT Kit (Qiagen, Netherlands) according to manufacturer's instructions. Initial denaturation of template RNA was done at 65°C for 5 minutes before added into the master mix [1X Buffer RT, 0.5 mM dNTPs, 1.0 µM of each primer, 500.0 ng/µl RNA and 4.0 units/µl Omniscript Reverse Transcriptase]. RT cocktail was then allowed to react at 37°C for 2 hours before proceeding to amplification of OmpC cDNA. Lastly, targeted transcripts were analysed via 1% (w/v) agarose gel electrophoresis, along with non-RT controls to trace for any potential DNA contamination.

Evaluation of Protein Expression

Extraction of soluble protein from infiltrated leaves

was done as previously described [22]. Following grinding of 200 mg leaf tissues, samples were suspended in 1X volume of Extraction Buffer [50 mM Tris-HCl at pH 7.25, 150 mM NaCl, 2 mM EDTA and 0.1% (v/v) Triton X-100]. After high-speed centrifugation (14,000 rpm) at 4°C for 10 minutes, the supernatant was collected for quantification using Pierce® 660 nm Protein Assay Reagent (Thermo Scientific, USA) prior storage at -80°C. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed at 120 V for 90 minutes to check the state of protein integrity. The 12% separating gel was stained with Coomassie blue and then analysed under GS-800™ calibrated densitometer (Bio-Rad, USA). For Western blot analysis, 20 µg/ml of protein extract was loaded in each well and subsequently electroblotted onto methanol-treated Polyvinylidene Difluoride (PVDF) membrane (MILIPORE, USA). Blocking step was done by incubating the membrane with 1X PBS-Tween (0.05 M Sodium Dihydrogen Phosphate at pH 7.4, 0.15 M NaCl and 0.05% Tween-20) supplemented with 4% (w/v) milk. Primary antibody used for detection was rabbit anti-His polyclonal antibody (Abcam, UK) (1:5,000 dilution). The mouse anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) monoclonal antibody (Thermo Scientific, USA) (1:1,000 dilution) was used as a loading control. After 2-hour shaking at 50 rpm, the membrane was washed and followed sequentially by 1-hour incubation of anti-rabbit HRP-linked secondary antibody (Abcam, UK) (1:10,000 dilution). Another washing step was repeated five times at 5-minute interval before 1 ml of 3, 3', 5, 5'-tetramethylbenzidine (TMB) substrate was added. Finally, the colorimetric response of the immunoblot was recorded using the same calibrated densitometer.

RESULTS AND DISCUSSION

Salmonella gallinarum Characterisation

Comparatively slower growth was observed from the smooth, domed and glistening salmonellae colonies. In addition, selective conditions of MacConkey and Xylose-Lysine-Deoxycholate agar plates had recorded the bacterial response as a non-lactose fermenter and hydrogen sulphide-negative enteric coliform. From the microscopic observation, the pinkish non-sporulating microbes were seen to be arranged as a single entity that conformed to the measured 1.0-2.5 µm length and 0.3-1.5 µm width [8]. Examination of *S. gallinarum* biochemical properties as summarised in Table 1 revealed catabolic breakdown of dextrose, maltose and mannitol which was indicated by phenol red

without any gas evolution (Figure 2a). Next, the colour change of bromocresol purple had denoted positive reaction in decarboxylase tube containing lysine only (Figure 2b). Evidence of bacterial non-locomotive feature was recorded based on the confined growth from the line of inoculation (Figure 2c). Nevertheless, a 7-digit code of 4004102 was generated based on the sheet attached with API kit.

Isolation of Antigenic Gene

Extraction of intact bacterial gDNA (data not shown) was successful, yielding two bands of ~2.5 kbp and >10 kbp in size on the agarose gel. Consecutively, amplification of the OmpC gene from the genomic template was achieved using designated primers. With the annealing temperature set at 67°C, ~1.002 kbp amplicons were obtained and thereafter named as OmpC-KDEL.

Recombinant Vector Construction

Recombinant vector, namely pEAQ::OmpC-KDEL was generated as illustrated in Figure 1b. The presence of the transgene in *E. coli* clones was verified by PCR (Figure 3). XmaI- and NruI- digested amplicons and pEAQ-HT with molecular sizes of ~0.988 kbp and ~9.973 kbp, were ligated through complementary sequences to yield the expression cassette (Figure 1b). Sequence alignment results had scored 99.8% of data

Table 1. A summary of *S. gallinarum* biochemical characteristics which is corresponding to Figure 2.

(a) Carbohydrate fermentation test	
Sugar media	<i>S. gallinarum</i> growth response
Control	No reaction
Dextrose	Phenol red turned yellow without gas formation
Maltose	Phenol red turned yellow without gas formation
Lactose	No reaction
Mannitol	Phenol red turned yellow without gas formation
Sucrose	No reaction
(b) Amino acid decarboxylation test	
Substrate	<i>S. gallinarum</i> growth response
Control	No reaction
Ornithine	No reaction
Lysine	Turned purple
Arginine	No reaction
(c) Motility or locomotive test	
Tube	Description
Control	No growth
<i>S. gallinarum</i>	Growth only along stabbed line of inoculation
<i>E. coli</i>	Diffused bacterial growth spreading throughout the tube

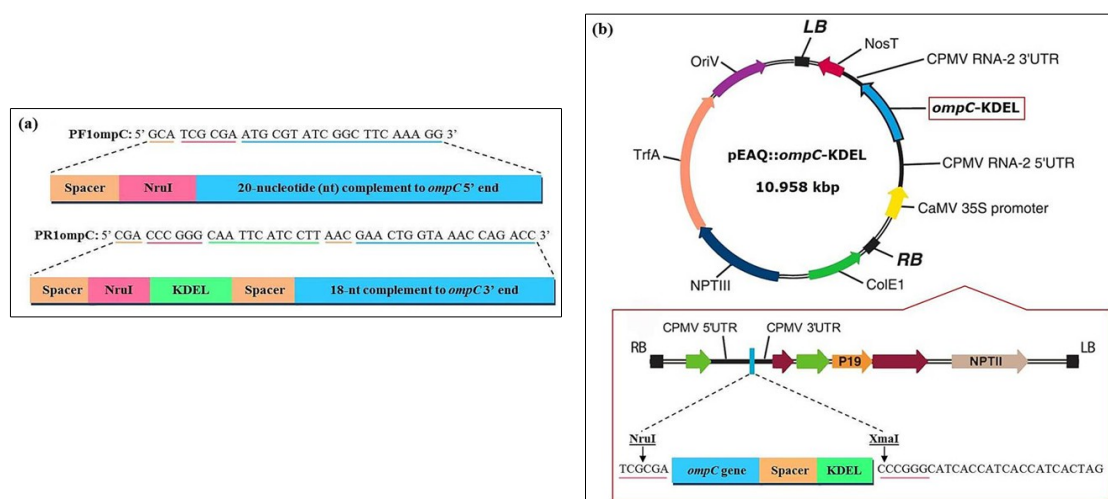


Figure 1. Schematic diagrams of (a) PF1ompC and PR1ompC primers designed for OmpC gene isolation, with additional sequences added onto 5' or 3' end; and (b) construction of gene expression cassette pEAQ::OmpC-KDEL, as adopted from Sainsbury et al. [35]).

similarity when BLAST analysis was performed against the Accession Number NC_011274.

Morphological Observation of Plants

Following agroinfiltration, a systemic spread of plant virus was noted; with abnormal morphologies like leaf chlorosis, crinkle and/or necrosis of tobacco host. Emission of green fluorescence was detected from pEAQ::GFP-infected leaves upon sample collection (Figure 4).

Heterologous Expression of OmpC Gene at RNA and Protein Levels

The RT-PCR profiles as depicted in Figure 5 confirmed successful expression of the antigenic gene. Bands with a molecular size of ~1.002 kbp were obtained for samples at 3, 6 and 9 dpi, conformed to the positive controls (lanes 13 and 28). Healthy and pEAQ-HT-infiltrated leaves did not show amplification

as expected. For preliminary testing, a positive signal was detected by dot blot assay of pEAQ::OmpC-KDEL-infiltrated samples (data not shown). Compatible hybridization was further verified via Western blot analysis (Figure 6a). The presence of OmpC protein at ~35 kDa (UniProtKB Database: B5RC93) was detected in all plant samples infected with pEAQ::OmpC-KDEL. Contrariwise, none was detected for pEAQ-HT-infected and healthy ones. Correspondingly, the presence of ~34 kDa housekeeping GAPDH protein was identified from all plant samples (Figure 6b).

In agreement to the general features of salmonellae, the avian-adapted *S. gallinarum* was identified as gram-negative non-sporogenic tiny rods that grew slowly on nutritive and selective media. In view of biochemical deficiency, an appreciable growth of the serotype could be limited by the auxotrophic requirement for thiamin, which was not usually formulated into customary

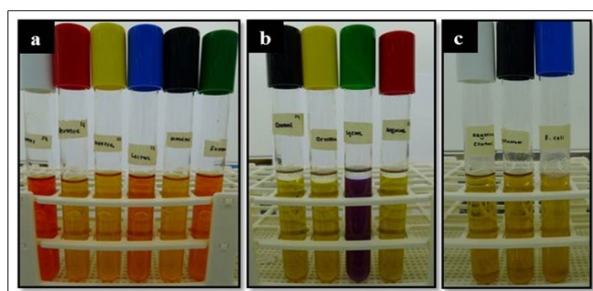


Figure 2. Biochemical tests, namely (a) carbohydrate fermentation, (b) amino acid decarboxylation; and (c) motility analyses in semi-solid agar were conducted to identify *S. gallinarum*.

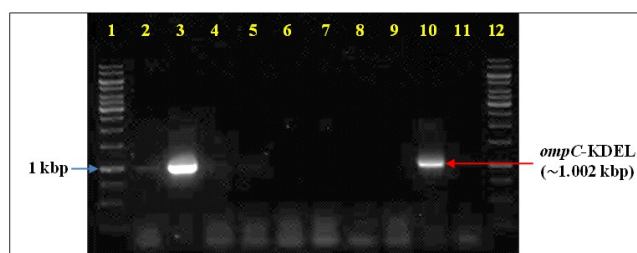


Figure 3. PCR screening profiles of XL-1 *E. coli* transformed colonies harbouring pEAQ::OmpC-KDEL. L1 and L12: GeneRuler™ 1 kbp DNA Ladder; L2-L9: PCR products amplified from kanamycin-resistant colonies; L10: Positive control of OmpC amplicon; L11: Negative control

bacteriological media [24]. Based on the carbohydrate fermentation test, catabolic breakdown of dextrose, maltose and mannitol into acids were displayed by *S. gallinarum*. Interestingly, no formation of gas bubbles was detected as compared to *E. coli*. Despite that, results obtained did conform to other findings [25, 26]. Vast biochemical similarities between the two avian-specific serovar Gallinarum and serovar Pullorum had been ascribed to their monophyletic divergence. Hence, ornithine decarboxylation test was incorporated to validate the identity of *S. gallinarum* due to its naturally mutated speC gene that blocks putrescine biosynthesis [25,27]. The credibility of data on ornithine and lysine decarboxylation was in fact strengthened by previous report [28]. However, a discrepancy was noted for arginine decarboxylase activity as the pathway had been discovered previously [27]. Repetitive tests were done and it could be narrowed down to two factors: (i) pH shift induced by arginase, arginine dihydrolase and diamine oxidase or (ii) widespread interchange of genetic elements between compatible strains given the diversity among isolates [29-31]. Besides, a locomotive test was crucial to distinguish salmonellae into serotypes of paratyphoid and avian specific. The absence of peritrichous flagella in the latter group could due to

the faulty assemblage of flagellar subunits or dysfunctional motor proteins [30].

Guided by concrete evidences of OmpC as immunogen [32], a pioneering study was established here to present the reproducibility of plant-based transient expression as a time- and cost-effective platform in developing a vaccine candidate against fowl typhoid. The binary vector pEAQ-HT has built-in CaMV35S promoter (P-35S) and Nos terminator (NosT) flanking between the T-DNA borders to mediate high OmpC-KDEL expression. The presence of p19 silencing suppressor is expected to promote expression through sequestration of small-interfering RNAs generated by Dicer [33]. Considering the plant protein glycosylation pattern, KDEL insertion was projected to minimise protein conformational changes that would impact neutralising antibodies production. Accumulation of ER-resident proteins could benefit the yield harvest attributed to 20-100 folds of improved expression, greater stability and minimised exo-proteinase degradation in cytoplasm [34].

BLASTn had recorded 99.8% of maximum identity and an E-value of 0, indicating that the similarity score was significant to prove that sequences were homologs. In other words, convincing evidence of successful re-

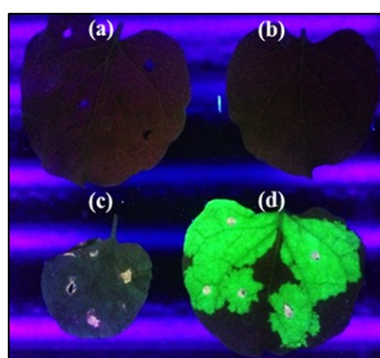


Figure 4. Detection of green fluorescence under an ultraviolet box. The expression of GFP protein was compared between (a) pEAQ-HT-infected (mock control), (b) healthy tobacco, (c) pEAQ::OmpC-KDEL-infiltrated and (d) pEAQ::GFP-infected (positive control) leaves as a checkpoint for successful agroinfiltration.

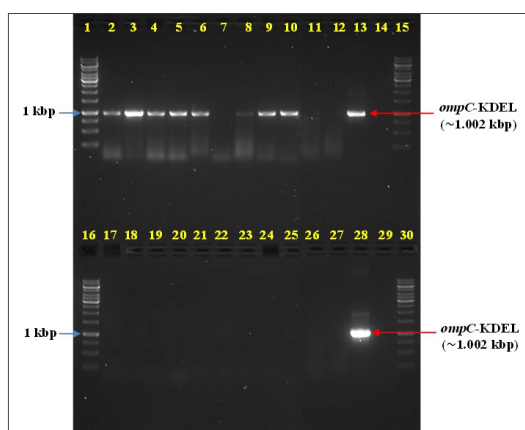


Figure 5. RT-PCR amplification profiles of RNA extracted from infiltrated *N. benthamiana* leaves [L1, L15, L16 and L30: GeneRulerTM 1 kbp DNA Ladder; L2-L10: Triplicates of pEAQ::OmpC-KDEL-infected plant samples at 3, 6 and 9 dpi; L17-L25: Corresponding non-RT controls for transgene; L11 and L26: pEAQ-HT-infiltrated leaf and its non-RT control; L12 and L27: Healthy tobacco and its non-RT control; L13 and L28: PCR positive controls; L14 and L29: PCR negative controls]. Expected ~1.002 kbp OmpC-KDEL amplicons were obtained, conforming to the band size of PCR positive control.

combinant DNA construction was achieved. A transient expression assay was opted here to assess the capacity of plant system to express the foreign antigenic sequence preceding stable transgenesis [35]. Based on the RT-PCR profiles, amplicons of OmpC-KDEL (~1.002 kbp) were obtained at all days tested, signifying positive expression at the transcriptional level. The possibility of OmpC-KDEL expression being affected by individual host's physiological condition could not be excluded as low transcript abundance was also noted (Figure 5, lanes 7 and 8). Only samples of 6 dpi were selected for subsequent immunoblotting since OmpC transcripts at this time point were consistently detected. Compatible hybridization between His-tagged OmpC and anti-His antibody through western blotting assay had further confirmed successful expression of recombinant antigen where an expected band size of ~35 kDa was obtained only in pEAQ::OmpC-KDEL-infiltrated samples. Besides, protein bands of 50 kDa

were postulated to be background proteins of tobacco host that reacted with the anti-His antibody (data not shown). As the Ribulose-1, 5- biphosphate carboxylase/oxygenase (Rubisco) constitutes at least 50% of the total soluble protein in C3 plants [36], it is likely that the 50 kDa product represents the degrading fragment of Rubisco large subunit. Turnover could be prompted by oxidative stress suffered during CPMV-based infection. Nevertheless, solid detection of OmpC up to protein stage was achieved. It is also interesting to note that satisfactory OmpC expression could be achieved even without codon optimisation of the native bacterial sequence.

Nevertheless, His-tag detection could not serve as a definite resemblance of antibody hybridization directed specifically against *S. gallinarum* OmpC. Also, because a polyclonal anti-His was used, it could not confer high stringency like monoclonal antibody that only binds to single epitopes. Thus, this would limit the detection of

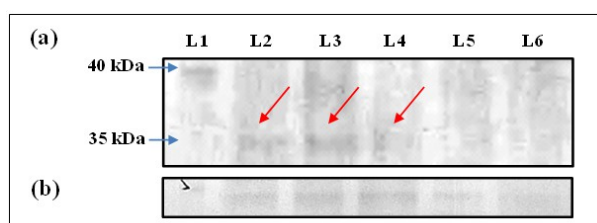


Figure 6. Immunoblotting profiles of protein samples extracted from infiltrated *N. benthamiana* leaves at 6 dpi reacted with (a) anti-His and (b) anti-GAPDH antibodies. L1: Spectra™ Multicolor Broad Range Protein Ladder; L2-L4: Replicates of pEAQ::OmpC-KDEL-infected plant samples; L5: Healthy tobacco; L6: pEAQ-HT infiltration control. The OmpC protein was successfully detected at ~35 kDa region for all samples infiltrated with pEAQ::OmpC-KDEL (red arrows).

low OmpC expression by which merely fade bands were obtained along with non-specific interference by most probably Rubisco. Albeit His-tagged fusion was valued for downstream purification capacity, the impracticality of the assay to detect poorly expressed protein, however was reported [37], attributed to interfering background and length of the procedure. Enrichment procedure could be employed to pool eluents of His-tagged protein from nickel affinity column prior purifying via high-performance liquid chromatography to enhance the performance of anti-OmpC-based detection [38].

CONCLUSIONS

In this study, rapid biochemical identification of *S. gallinarum* clinical isolate along with successful expression of OmpC-KDEL in *N. benthamiana* signify its momentous value as a potential plant-based vaccine development against emerging fowl typhoid. The high degree of sequence homology between *Salmonella* porins further enhances the practicality of recombinant OmpC in conferring protection against other infectious serotypes of significant importance in poultry industries. Nevertheless, confirmatory hybridization of anti-OmpC against the immunogen is proposed to achieve more comprehensive study for functional validation in future. Once the immunogenic protection is proven to be promising, this research could then be exemplified as a platform for the transformation of palatable plant species such as alfalfa and corn to function as an edible vaccine.

Comprehensive scope which includes clinical strain identification, antigenic protein extraction and OmpC expression studies had highlighted the robustness of the transient technology in delivering pandemic vaccines within the shortest time frame. Considering from the production standpoint, the advantages of speed and cost would vastly benefit from the instantaneous supply of potent vaccines to global market prior waves of any further epidemic loss.

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